DATA EVALUATION RECORD

CAPHRA/BIFENTHRIN

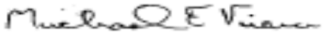
Study Type: OCSPP Non-Guideline; *In Vitro* Metabolism Kinetics

EPA Contract No. EP-W-16-018
Task Assignment No.: 32-3-034 (MRID 50803904)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

3201 Jermantown Rd., Suite 400
Fairfax, VA 22030

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/21/2019
Secondary Reviewer: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/24/2019
Quality Assurance: Sarah E. Saucier, Ph.D.	Signature: Date:	 05/30/2019
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/31/2019

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel**.** Contractor's role did not include establishing Agency policy.

EPA Reviewer: Evisabel Craig, Ph.D., DABT **Signature:** E.C. Craig
Risk Assessment Branch VI, HED (7509C) **Date:** 8/1/2019
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DATA EVALUATION RECORD

STUDY TYPE: OCSPP Non-guideline; *In Vitro* Metabolism Kinetics.

PC CODE: 128825

DP BARCODE: D451266

TXR#: 0057896

TEST MATERIAL (PURITY): Bifenthrin (95.9% a.i.)

SYNONYMS: (2-methyl[1,1′-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate

CITATION: Brown, S. (2019) Bifenthrin: A study to determine the kinetics of metabolism of bifenthrin in selected expressed human carboxylesterase (CES) and cytochrome P450 (CYP) enzymes; final report. Concept Life Sciences Dundee, Dundee Technopole, Dundee, United Kingdom. Laboratory Project ID: CXR1723-IV bifenthrin, January 10, 2019. MRID 50803904. Unpublished.

SPONSOR: Council for Advancement of Pyrethroid Human Risk Assessment, LLC (CAPHRA), c/o Household and Commercial Products Association, 1667 K Street, NW, Suite 300, Washington DC

EXECUTIVE SUMMARY: In a non-guideline, *in vitro* metabolism study (MRID 50803904), the apparent intrinsic clearance (CL_{int}) of bifenthrin (95.9% a.i.; Lot # PL15-0330) was determined in recombinant microsomes. The enzymes expressed by these systems were CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CES1b, and CES2 (see Appendix 1 at the end of this DER for product details); a control system also was included. For the CYP enzymes, preliminary experiments with were conducted with bifenthrin to screen for metabolism by the enzyme variants. Duplicate incubations were conducted with the CYP enzymes (10 pmol/mL) and bifenthrin (0.1 µM) with a minimum protein concentration of 0.1 mg/mL achieved by the addition of control microsomes. Incubations with control microsomes at the same concentrations as the CYP microsomes were performed to correct for endogenous CES activity. If the total rate (k_{dep}) for the CYP enzyme was greater than twice the rate of the control microsomes, it was selected for further experimentation. For the CES enzymes, no preliminary experiment was conducted due to the lack of bifenthrin metabolism in the presence of CES enzymes observed in a previous investigation (CXR1722; no further data provided). The main study was conducted in duplicate with CYP1A2, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, CYP3A5, CES1b, and CES2 in the presence of NADPH (CYP only) with 0.1 µM bifenthrin. Two experiments were conducted with the data from the first experiment used for selection of time

points used in the second experiment (a third confirmatory experiment was conducted for CYP3A4, CYP3A5, and CYP2C8). Incubations with bifenthrin at 0.1 µM in CES1b (100 pmol/mL) and CES2 (200 pmol/mL) enzyme preparations also were conducted in duplicate on two separate occasions with incubation times of 2 h. The final concentrations of bifenthrin were determined by LC/MS.

Rates of depletion were determined from plots of the natural logarithm of the percentage of bifenthrin remaining against incubation time by linear regression. Estimates of CL_{int} for CYP and CES enzymes were calculated with the following equation:

$$CL_{int} = \frac{0.693}{t½} \times \frac{\text{Incubation volume (mL)}}{\text{pmol CYP or CES}}$$

RESULTS

PRELIMINARY EXPERIMENTS: Duplicate incubations with 0.1 µM bifenthrin in recombinant human microsomes were conducted in the presence of NADPH. Incubation times generally were not reported but it was stated that because the CL_{int} for CYP1A2 could not be calculated from the data generated during the first incubation due to insufficient loss (May 11; 30-min incubation), two sets of 2-h incubations were included for the second incubation (May 17). Preliminary results for the CYP enzymes were presented in Table 1 on page 20 of MRID 50803904 and are included in Appendix 2 at the end of this DER. CYP1A2, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, and CYP3A5 metabolized bifenthrin at ≥4.48× the rate of control microsomes and were selected for the main study experiments.

MAIN STUDY EXPERIMENTS: Results for the main study experiments were presented in Tables 2 (CYP) and 3 (CES) on pages 21 and 22, respectively, of MRID 50803904 and are included in Appendix 3 at the end of this DER. Estimates of bifenthrin Cl_{int} were calculated for CYP2C19, CYP3A4, CYP2C8, CYP3A5, and CYP2C9*1. Because there was a three-fold difference in calculated CL_{int} values for metabolism in CYP3A4 between the first and second experiments conducted on May 11 and 17, respectively, a third experiment was conducted. Because the results from the third experiment (June 6) were similar to those from May 11, the data from May 17 were not used in the calculation of the mean values. Additionally, there was a 1.8-fold difference in calculated CL_{int} values for metabolism in CYP2C8 between the first and second experiments conducted on May 11 and 17, therefore, a third experiment was conducted. Because the results from the third experiment (June 6) were similar to those from May 11, the data from May 17 also were not used in the calculation of the mean values. Because a CL_{int} value could not be calculated for CYP3A5 from the data generated on May 17, a third experiment was conducted on June 6.

Bifenthrin was found to be metabolized by CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, and CYP3A5 expressed enzymes. The highest CL_{int} value was observed for CYP2C19. CYP2C19 metabolized bifenthrin at a rate approximately 49-fold greater than CYP2C9*1 and the Cl_{int} was approximately 23-fold greater than CYP3A4 (the second highest estimated CL_{int}). Bifenthrin was not metabolized by the expressed CES enzymes (CES1b or CES2).

Estimated CL_{int} values ranged from 0.243 µL/min/pmol for CYP2C9*1 to 11.8 µL/min/pmol for CYP2C19; no metabolism was observed for CYP1A2 or CYP2B6. In addition, no detectable metabolism of bifenthrin during 2-h incubations was observed with CESlb (100 pmol/mL) or CES2 (200 pmol/mL).

REVIEWER'S COMMENTS: This is a non-guideline study and was submitted as part of CAPHRA's effort to assess the pharmacokinetic properties of the pyrethroids.

APPENDIX 1

Source of test systems: Recombinant human "supersomes" were purchased from Corning B.V. Life Sciences, Amsterdam, The Netherlands. These "supersomes" are microsomes having recombinant human enzymes expressed by baculovirus-infected insect cells (see table below).

Expressed enzyme	Product number	Lot Number	Expressed enzyme	Product number	Lot Number
CYP1A2	456203	7320001	CYP3A4	456202	5224002
CYP2A6	456254	5315001	CYP3A5	456256	5258001
CYP2B6	456255	5239002	CYP3A7	456237	5246004
CYP2C8	456252	7255002	CYP4A11	456221	5266001
CYP2C9*1	456258	6257001	CES1b	453320	6230004
CYP2C19	456259	7262001	CES2	453322	6084004
CYP2D6*1	456217	456217	Control	456244	6180001
CYP2E1	456206	5265003			

(copied from page 11 of MRID 50803904)

APPENDIX 2

Expressed enzyme	Date	Bifenthrin conc µM	Total K_{dep} min^{-1}	Control K_{dep} min^{-1}
CYP2C19	23-Mar-18	0.1	0.223	0.000298
CYP3A4	26-Mar-18	0.1	0.00335	0.000648
CYP3A5	26-Mar-18	0.1	0.00240	0.000453
CYP2C9*1	23-Mar-18	0.1	0.00229	0.000189
CYP2C8	23-Mar-18	0.1	0.00221	0.000189
CYP2D6*1	26-Mar-18	0.1	0.00107	0.000648
CYP1A2	23-Mar-18	0.1	0.00106	0.000189
CYP3A7	26-Mar-18	0.1	0.00103	0.000648
CYP4A11	26-Mar-18	0.1	0.000861	0.000936
CYP2B6	23-Mar-18	0.1	0.000846	0.000189
CYP2E1	26-Mar-18	0.1	0.000517	0.000648
CYP2A6	23-Mar-18	0.1	0.000388	0.000189

(copied from page 20 of MRID 50803904)

APPENDIX 3

Expressed Enzyme	Date	Bifenthrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CYP	Mean CL_{int} µL/min/pmol CYP
CYP2C19	11-May-18	0.1	0.124	5.6	12.4	11.8
	17-May-18	0.1	0.111	6.2	11.1	
[1]CYP3A4	11-May-18	0.1	0.00567	122.4	0.567	0.507
	17-May-18	0.1	0.00184	377.3	0.184	
	06-Jun-18	0.1	0.00447	155.2	0.447	
[2]CYP2C8	11-May-18	0.1	0.00404	171.6	0.404	0.432
	17-May-18	0.1	0.00227	304.9	0.227	
	06-Jun-18	0.1	0.00459	151.0	0.459	
[3]CYP3A5	11-May-18	0.1	0.00278	249.9	0.278	0.260
	17-May-18	0.1	CL_{int} not calculated			
	06-Jun-18	0.1	0.00243	285.9	0.243	
CYP2C9*1	11-May-18	0.1	0.00414	167.4	0.414	0.243
	17-May-18	0.1	0.00243	285.9	0.243	
CYP2B6	11-May-18	0.1	No metabolism detected			
	17-May-18	0.1				
CYP1A2	11-May-18	0.1	No metabolism detected			
	17-May-18	0.1				
	17-May-18	0.1				

[1] Data from the CYP3A4 experiment on 17 May 2018 should not be used, and have not been included in the calculation of means, as there is a notable difference in calculated CL_{int} between this experiment and those of 11 May 2018 and 06 June 2018.

[2] Data from the CYP2C8 experiment on 17 May 2018 should not be used, and have not been included in the calculation of means, as there is a notable difference in calculated CL_{int} between this experiment and those of 11 May 2018 and 06 June 2018.

[3] CL_{int} for CYP3A5was unable to be calculated from the data generated on 17 May 2018.

Rates of bifenthrin depletion in control supersomes were subtracted from the total rate to correct for endogenous CES activity.

(copied from page 21 of MRID 50803904)

Expressed Enzyme	Date	Bifenthrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CES	Mean CL_{int} µL/min/pmol CES
CES1b 100 pmol/mL	29-May-18	0.1	No metabolism detected			
	31-May-18	0.1				
CES2 200 pmol/mL	29-May-18	0.1	No metabolism detected			
	31-May-18	0.1				

Rates of bifenthrin depletion in BSA were subtracted from the total rate to correct non specific loss of bifenthrin.

(copied from page 22 of MRID 50803904)